26:1691). Counting of transcripts at each DNA template suggested a stochastic initiation mechanism in the experimental system. We found a prototypical activator (human Sp1) regulates transcription by enhancing PIC assembly (presumably by recruiting TFIID). Real-time TFIID binding to DNA was monitored and coupled to transcription detection at the same DNA template for the first time. We also developed methods to detect the production of RNA transcripts in real-time and couple that to the kinetic measurements of RNA polymerase binding at the single-molecule level. using multiple fluorescently labeled General Transcription Factors (GTFs, namely TFIIB TFIID, TFIIE, TFIIF and TFIIH) and Pol II, we are currently investigating the structure of PIC, pathways of its assembly, and the mechanism of transcription modulation by sequence-specific activators and the core promoter DNA elements.

Symposium: Awards Symposium

1873-Symp
Cholesterol Nanodomains: their Effect on Monolayer Morphology and Dynamics
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Small mole fractions of cholesterol segregate into 10 - 100 nm diameter nanodomains in dipalmitoylphosphatidylcholine (DPPC) monolayers. The nanodomains segregate to DPPC domain boundaries, reducing the line tension, Δ, which alters domain shapes. The nanodomains consist of a 6:1 lipid: cholesterol ''complex'' and the surface viscosity, ηs, decreases exponentially with the area fraction of the complex at a given surface pressure. ηs increases exponentially with surface pressure, independent of cholesterol content, as predicted by a free area model that relates ηs to monolayer compressibility and collapse pressure. G', the elastic modulus, decreases with Δ at low cholesterol fractions, in analogy to 3-D emulsions. Increasing cholesterol further causes a sharp increase in G', likely due to a transition from a tilted to untilted molecular packing. Understanding the effects of small mole fractions of cholesterol should resolve the role of cholesterol in human lung surfactants and may give clues as to how cholesterol influences raft formation in cell membranes.

Platform: Ca-activated Channels

1874-Plat
Tmem16F forms a Ca^{2+}-Activated Cation Channel Required for Lipid Scrambling in Platelets during Blood Coagulation
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Collapse of membrane lipid asymmetry is a hallmark of blood coagulation. TMEM16F of the TMEM16 family that includes TMEM16A/B Ca^{2+}-activated Cl- channels (CaCCs) is linked to Scott Syndrome with deficient Ca^{2+}-dependent lipid scrambling. We generated TMEM16F-knockout mice that exhibit bleeding defects and protection in an arterial thrombosis model associated with platelet deficiency in Ca^{2+}-dependent phosphatidylserine exposure and procoagulant activity, and lack a Ca^{2+}-activated cation current in the platelet precursor megakaryocytes. Heterologous expression of TMEM16F generates a novel Small-conductance Ca^{2+}-Activated Non-selective cation (SCAN) current with sub-picosiemens single channel conductance rather than a CaCC. TMEM16F-SCAN channels permeate both monovalent and divalent cations including Ca^{2+}, and exhibit synergistic gating by Ca^{2+} and voltage. We further pinpointed a residue in the putative pore region important for the cation versus anion selectivity of TMEM16F-SCAN and TMEM16A-CaCC channels. This study thus identifies a novel Ca^{2+}-activated channel permeable to Ca^{2+} and critical for Ca^{2+}-dependent scramblase activity.

1875-Plat
Ca^{2+}-Dependent Ion and Lipid Transport Mediated by a Fungal TMEM16 Homologue
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Ca^{2+}-activated Cl- channels (CaCCs) play crucial roles in human physiology, from epithelial secretion to nociception and sensory transduction. Recent work showed that two members of the TMEM16 family, TMEM16A and B, encode for CaCCs. The TMEM16 family is comprised of 10 human homologues whose malfunction has been implicated in several human diseases. Despite their physiological relevance the function of the other TMEM16s is unclear and controversial. For example, TMEM16F has been reported to be a Ca^{2+}-dependent cation channel, three different Cl- channels and to be involved in lipid scrambling (lipid transport between membrane leaflets). These data raise the possibility that not all TMEM16 proteins are CaCCs and that some might be scramblases or regulators of scrambling activity.
To differentiate between these hypotheses we expressed, purified and reconstituted several TMEM16 family members and discovered that a fungal homologue simultaneously mediates ion movement and lipid scrambling. Reconstitution in planar lipid bilayers shows a non-selective ion channel of high conductance, ~300 pS. Both transport functions are tightly regulated by Ca^{2+}: in its absence ion channel activity is abolished and lipid scrambling is severely diminished. The apparent Km of Ca^{2+} for transport is ~400 nM, a value comparable to that of other TMEM16s. We mutated a highly conserved di-acidic motif previously shown to be important for Ca^{2+}-regulation in TMEM16A and F. Simultaneous charge-neutralization of these residues eliminates Ca^{2+} dependent activation of ion and lipid transport, suggesting that a single Ca^{2+} site regulates both.
Our results demonstrate for the first time that a member of the TMEM16 family is simultaneously a Ca^{2+} dependent ion channel and a Ca^{2+} dependent lipid scramblase. This suggests that other family members, such as TMEM16F, might also be dual function proteins thus resolving the confusion regarding their function.

1876-Plat
Leukotriene B4 Activation of Arterial Smooth Muscle BK Channels
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Blood circulation depends on the myogenic tone of small arteries. Tone is increased by a rise in overall cytosolic calcium $[Ca^{2+}]_i$ in the arterial smooth muscle cell (SMC). This rise leads to activation of large-conductance, Ca^{2+}/voltage-gated K^+ (BK) channels. This generates outward currents that hyperpolarize the membrane and limit Ca^{2+} entry. Thus, BK channel activation opposes SMC contraction.
Several negatively charged endogenous lipids, such as PIP_2, fatty and bile acids have been shown to directly activate SMC BK channels. The chemical structure of leukotrienes (LTs) includes a long hydrophobic backbone and a carboxylic end group, which is largely ionized at physiological pH=6.8-7.4. Thus, we hypothesized that LTs were BK channel activators.
We tested LTA4, LTB4, LTC4, LTD4, and LTE4 on heterologously expressed BK channels conformed by cbv1 (AY330293) and rβ1 (FJ154955) subunits cloned from rat cerebral artery SMC. LTs were applied at 1 nM to the cytosolic side of inside-out (I/O) membrane patches from *Xenopus* oocytes; Vm=-80 to −20 mV, $[Ca^{2+}]_i$=10 μM. LTB4 was the only LT that significantly increased BK channel activity (130% from pre-LT values), with a ≈330% increase in activity in response to 2 μM LTB4. LTB4-induced BK channel activation was also observed in I/O patches from freshly isolated cerebral artery SMCs. Therefore, we identified LTB4 as a potent and effective SMC BK channel activator that works independently of cell signaling. using pressurized, de-endothelized cerebral arteries from rats, we blocked LTB4 production by 50 μM bestatin. This treatment reduced artery diameter by ≈5%, which is expected to cause up to 10-20% decrease in cerebral blood flow. This result suggests that LTB4 production within arterial SMC exerts a tonic effect on arterial tone, favoring SMC relaxation and opposing vasoconstriction.

1877-Plat
Maxik Interaction with Gaba Transporter 3 and Heat Shock Protein 60 in the Mouse Brain
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Large conductance voltage- and calcium-activated potassium (MaxiK) channels regulate Ca^{2+} signaling, transmitter release, and repolarization of the action potential in neurons. Analysis of MaxiK interactome from the mouse brain using liquid chromatography/mass spectrometry in tandem and knockout animals, as negative controls, revealed that MaxiK interacts with a variety of proteins localized to the mitochondria, plasma membrane, cytoplasm, Golgi apparatus, endoplasmic reticulum and other intracellular organelles. We examined whether proteins identified by mass spectrometry colocalize with MaxiK channel in cells. To this end, we have first chosen two proteins, namely gamma-aminobutyric acid (GABA) transporter (GAT)-3, and a heat shock protein

(HSP)-60. GAT-3 is expressed in unmyelinated axons and glial processes, whereas HSP-60 is present in the cytosol and mitochondria, and is implicated in translocation of mitochondrial proteins from the cytoplasm. Immunocytochemical studies in HEK293T cells showed that MaxiK colocalizes with GAT-3 at the plasma membrane and HSP60 at the cell periphery. These results indicate that MaxiK could be playing a role in modulating GABA release from the presynaptic nerve terminals via GAT-3, and HSP-60 could be involved in translocating MaxiK to the mitochondria. Supporting the latter, we have confirmed the presence of MaxiK in isolated brain mitochondria using immunocytochemistry. Further studies will help to understand the role of MaxiK in modulating GAT-3 or vice versa and the role of HSP-60 in targeting MaxiK to brain mitochondria. Supported by AHA and NIH.

1878-Plat

External Architecture of the Large-Conductance Ca^{2+} and Voltage-Activated K^+ (BK_{Ca}) Revealed by a Spectroscopic Ruler

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BK_{Ca} channels are involved in a large variety of physiological processes and regulatory β subunits are one of the mechanisms responsible of creating BK_{Ca} channel diversity fundamental to the adequate function of many tissues. Regardless the proven importance of this channel little is known about its detailed structure. Here we disclose the external architectural intimacies of BK_{Ca} channels using Lanthanide based Resonance Energy Transfer (LRET) as a molecular ruler to measure intra and intermolecular distances. We introduced a genetically encoded lanthanide binding tag (LBT that binds Tb^{3+} with high affinity) at several positions of the external loops of the α and β1 subunits, and constructed a fluorescent molecule of BODIPY-FL linked to a scorpion toxin, iberiotoxin (Bodipy FL-IbTX), that was used as an acceptor for the LRET interaction with Tb^{3+}. These functional LBT-BK_{Ca} constructs were expressed in *Xenopus laevis* oocytes that were voltage clamped with two microelectrodes to obtain simultaneously electrical and LRET recordings under physiological ionic conditions. Sensitized emission (SE) recordings from different LBT-BK_{Ca} positions had different kinetics indicating different relative positions for each construct. We analyzed SE records with a novel method developed by our group that determines the position of LBT-tagged sites of BK_{Ca} to obtain an external structural map, including the β1 subunit. Interestingly, when the BK_{Ca} α subunit was co-expressed with the regulatory β1 subunit, SE becomes slower, indicating a large conformational change of the BK_{Ca} channel structure. The methodology presented here gives us the first glimpses to the BK_{Ca} channel external surface structure in its different functional states with and without the β1 subunit. Supported by Fondecyt grant 1110430 and NIH grants U54GM087519 and GM030376. CINV is a Scientific Millennium Institute.

1879-Plat

Crystal Structures of the MthK RCK Domain Reveal Allosteric Interactions Among Calcium Binding Sites

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Regulator of K^+ conductance (RCK) domains form a conserved class of modulatory domains that undergo conformational changes with binding of metal cations and other ligands, to control gating of channels and transporters. In MthK, a prototypical RCK-containing K^+ channel, each of the channel's eight RCK domains binds multiple Ca^{2+} ions to reach the fully-activated state, which can give rise to a complex conformational trajectory. Here we present crystal structures of the MthK RCK domain bound with Ca^{2+} in a series of singly-, doubly-, and triply-liganded states. These structures begin to reveal local conformational changes in this RCK domain that may arise from binding of Ca^{2+} at individual sites and pairs of sites over a range of ionic conditions, providing insight toward interactions among the sites that may modulate channel gating. Crystals formed at low to moderate [Ca^{2+}] show Ca^{2+} bound only at a single site, termed C1, determined by residues D184, E210, and E212. In contrast, high [Ca^{2+}] (in otherwise identical conditions) results in a new crystal form, with Ca^{2+} bound at sites C1, C2 (near residues E248 and E266), and C3 (residues D305 and E326). The mutation D184N, which abolishes Ca^{2+} binding at C1, permits Ca^{2+} binding at C3 with moderate [Ca^{2+}], suggesting that Ca^{2+} binding at C1 inhibits binding at C3. This apparent negative coupling between sites C1 and C3 can be alleviated by the mutation E212Q, which permits Ca^{2+} binding at both C1 and C3 and facilitates Ca^{2+}-dependent activation. These results suggest a structural basis for allosteric interactions that, in turn, modulate Ca^{2+} dependent gating of the MthK channel.

1880-Plat

Targeting the Channel-Calmodulin Interface of Small-Conductance Ca^{2+}-Activated Potassium Channels

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Small- and intermediate-conductance Ca^{2+}-activated potassium channels, activated by Ca^{2+}-bound calmodulin, play an important role in regulating membrane excitability. These channels are also linked to clinical abnormalities. A tremendous amount of effort has been devoted to developing small molecule compounds targeting these channels. However, these compounds often suffer from low potency and lack of selectivity, hindering their potentials for clinical use. A key contributing factor is the lack of knowledge of the binding site(s) for these compounds. Here we report our discoveries of the binding pocket for the compounds of the 1-EBIO class, located at the calmodulin-channel interface, by X-ray crystallography. Mutations of the channel, based on the structure data and molecular docking, can effectively change the potency of these compounds. Our results provide insight into the molecular nature of the binding pocket and its contribution to the potency and selectivity of the compounds of the 1-EBIO class.

1881-Plat

Calcium Concentration Fluctuations and Subspace Volume Influence Calcium-Regulated Calcium Channel Gating and Subspace Dynamics

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Cardiac myocyte calcium signaling is often modeled using deterministic ordinary differential equations (ODEs) and mass-action kinetics. However, spatially restricted ''domains'' associated with calcium influx are small enough that local signaling may involve 1-100 calcium ions. Therefore, the question arises: is it appropriate to model the dynamics of subspace calcium using deterministic ODEs or, alternatively, do we require stochastic descriptions that account for the fundamentally discrete nature of these local calcium signals? To address this question, we constructed a minimal Markov model of a calcium-regulated calcium channel and associated subspace. We compared the expected value of subspace calcium concentration and channel open probability (a result that accounts for the small subspace volume and concentration fluctuations) with the corresponding deterministic model (an approximation that assumes large system size and ignores concentration fluctuations). When subspace calcium did not regulate calcium influx, the deterministic and stochastic descriptions agreed. However, when calcium-binding altered channel activity in the model, the continuous deterministic description often deviated significantly from the discrete stochastic model, unless the subspace volume is unrealistically large and/or the kinetics of the calcium binding are sufficiently fast, demonstrating that the calcium concentration fluctuations and subspace volume influence channel gating and subspace dynamics. This principle was also demonstrated using a physiologically realistic model of calmodulin regulation of L-type calcium channels introduced by Yue and coworkers [Tadross, Dick, Yue. Cell 133: 1228-40, 2008]. Additional work will consider the influence of slow and rapid buffers present in the subspace and whether and under what conditions these buffers mitigate the effects of concentration fluctuations on channel gating and subspace dynamics.

Platform: DNA Replication, Recombination, and Repair

1882-Plat

Direct Observation of Stalled Fork Restart and Lesion Bypass via Fork Regression in the T4 Replication System

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The restart of a stalled replication fork is a major challenge for DNA replication. Depending upon the nature of the damage, different repair processes might be triggered; one is template switching that is bypass of a leading strand lesion via a Holliday junction formed by fork regression. using Magnetic Tweezers (MT) to study the T4 bacteriophage enzymes, we have reproduced *in vitro* the complete process of template switching. We show that the UvsW DNA helicase in cooperation with the T4 holoenzyme can overcome leading strand lesion damage by a pseudo stochastic process periodically forming and